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- (71) **Applicant (for all designated States except US):** **BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM** [US/US]; 201 West 7th St., Austin, TX 78701 (US).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** **MILLS, Gordon, B.** [CA/US]; 1515 Holcombe Blvd., Unit 0950, Houston, TX 77030 (US). **JU, Zhenlin** [US/US]; 1515 Holcombe Blvd., Suite 1410, Houston, TX 77030 (US). **COOMBES, Kevin** [US/US]; 1515 Holcombe Blvd., Suite 1410, Houston, TX 77030 (US). **HENNESSY, Bryan** [IE/IE]; 9 Glen Easton Lawns, Leixlip Co., Kildare (IE).
- (74) **Agent:** **PARKER, David, L.**; Fulbright & Jaworski L.L.P., 98 San Jacinto Boulevard, Suite 1100, Austin, TX 78701 (US).

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Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

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(54) **Title:** METHODS FOR PREDICTING RECURRENCE RISK IN BREAST CANCER PATIENTS

(57) **Abstract:** The invention relates to methods and kits useful for predicting or assessing recurrence risk in a patient having breast cancer who has been administered or treated with an anti-estrogen drug. Protein marker panels and methods of using the expression levels of these protein markers in calculating cancer recurrence risk and likelihood of response to the treatment are provided.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/24133

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - G01N 33/53; C12Q 1/68 (2012.01) USPC - 435/7.1, 435/6.14; 436/64 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) USPC: 435/7.1, 6.14; 436/64 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC: 435/7.1, 6.1, 6.11, 6.12, 6.14; 436/64 (text search) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Electronic data bases: PubWEST (USPT, EPAB, JPAB, PGPB); Google Scholar Search terms: breast cancer, recurrence or relapse or progression free, biomarkers (e.g. CCNB1, PAI1, PR, BCL2, ER, GATA3), microarray, signature profile, proteomic, reverse phase protein array, chemotherapeutic regimen, tamoxifen, aromatase inhibitor, sulfatas		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y Y Y Y A	US 7,569,345 B2 (COBLEIGH et al.) 4 August 2009 (04.08.2009). Especially col 2 ln 1-4 and ln 29-45, col 3 ln 38 to col 4 ln 4, col 14 ln 32-34 and ln 57-60, col 23 ln 11-27. AHMAD et al. "Stromelysin 3: an independent prognostic factor for relapse-free survival in node-positive breast cancer and demonstration of novel breast carcinoma cell expression"; Am. J. Pathol.; March 1998; Vol. 152, No. 3; Pages 721-728. Especially abstract, pg 723 table 2, pg 724 fig 2 and table 4. US 2008/0108091 A1 (HENNESSEY et al.) 8 May 2008 (08.05.2008). Especially para [0099], [0100], [0106]. STANWAY et al. "Steroid sulfatase: a new target for the endocrine therapy of breast cancer"; Oncologist; April 2007; Vol. 12, No. 4; Pages 370-374. Abstract. US 2010/0209920 A1 (BAKER et al.) 19 August 2010 (19.08.2010)	1,2, 3, 5-13, 15, 18, 20, 21 ----- 14, 16, 17, 19 14 16, 19 17 1-3, 5-21
☐ Further documents are listed in the continuation of Box C. ☐		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 5 July 2012 (05.07.2012)		Date of mailing of the international search report 03 AUG 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/24133

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: claims 1-21, directed to a method for determining the likelihood of breast cancer recurrence after treatment or response to a treatment with an anti-estrogen drug in an individual, said method comprising: (a) measuring the protein expression levels of a panel of markers in a biological sample containing breast cancer cells obtained from the individual, wherein the panel of markers comprises:

- (i) CCNB1 and PAI1;
- (ii) CCNB1, PR and BCL2;
- (iii) CCNB1, PR, BCL2, and PAI1, or
- (iv) CCNB1, PR, BCL2, ER, and GATA3;

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-3 and 5-21; limited to CCNB1 and PAI1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

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Continuation of Box III: Lack of Unity of Invention

(b) calculating the recurrence risk score (RS) for said individual based on the expression levels of the markers in the panel; and
(c) using the RS to determine the likelihood of breast cancer recurrence or response to the treatment; wherein the first invention is limited to the first set of markers, CCNB1 and PAI1 (claims 1-3 and 5-21)(applicants may opt for additional markers to be searched by specifying the marker set and paying an additional invention search fee for each set of markers).

Groups II+: claims 22-27, directed to a kit for determining the likelihood of breast cancer recurrence after treatment or response to a treatment with an anti-estrogen drug in an individual, comprising a panel of antibodies selected from the group consisting of:

- 1) an antibody that specifically binds to CCNB1, and an antibody that specifically binds to PAI1;
- 2) an antibody that specifically binds to CCNB1, an antibody that specifically binds to PR, and an antibody that specifically binds to BCL2;
- 3) an antibody that specifically binds to CCNB1, an antibody that specifically binds to PR, an antibody that specifically binds to BCL2, and an antibody that specifically binds to PAI1; and
- 4) an antibody that specifically binds to CCNB1, an antibody that specifically binds to ER, an antibody that specifically binds to PR, an antibody that specifically binds to BCL2, and an antibody that specifically binds to GATA3; wherein the first invention is limited to option 1) an antibody that specifically binds to CCNB1 and an antibody that specifically bind to PAI1 (applicants may opt for additional antibody sets to be searched by specifying the antibody set and paying an additional invention search fee for each set).

The inventions listed as Groups I+ - II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the claims of Groups I+ and II+ are indicated in the Group descriptions, above. Groups I+ is not limited to the use of antibodies in the method of determining the likelihood of breast cancer recurrence, and includes the calculation of a risk score which is not shared by Groups II+.

Each of the subgroups shares in common the technical elements of the Group to which it belongs. However, these common technical elements do not represent an improvement over the prior art of US 7,569,345 B2 to Cobleigh et al., which teaches a method for determining the likelihood of breast cancer recurrence after treatment or response to a treatment with an anti-estrogen drug in an individual, said method, comprising:

(a) measuring the protein expression levels of a panel of markers in a biological sample containing breast cancer cells obtained from the individual (col 2 In 37-45; "In one aspect, the invention concerns a method of predicting the likelihood of long-term survival of a breast cancer patient without the recurrence of breast cancer, comprising determining the expression level of one or more prognostic RNA transcripts or their expression products in a breast cancer tissue sample obtained from the patient, normalized against the expression level of all RNA transcripts or their products in the breast cancer tissue sample"; col 14 In 57-60; "Proteomics methods are valuable supplements to other methods of gene expression profiling, and can be used, alone or in combination with other methods, to detect the products of the prognostic markers of the present invention"); wherein the panel of markers comprises:

(i) CCNB1 and PAI1 (col 3 In 38 continued to col 4 col 4 In ; "A multivariate stepwise analysis, using the Cox Proportional Hazards Model, was performed on the gene expression data obtained for all 79 patients with invasive breast carcinoma. The following ten-gene sets have been identified by this analysis as having particularly strong predictive value of patient survival; (n) BAG1, TP53BP2, PRAME, IL6, CCNB1, PAI1, AREG, tumor size, CA9, and Ki67");

(b) calculating the recurrence risk score (RS) for said individual based on the expression levels of the markers in the panel (col 11 In 11-27; "Multivariate analysis is performed using the following equation: $RR = \exp[\text{coef}(\text{geneA}) \times \text{Ct}(\text{geneA}) + \text{coef}(\text{geneB}) \times \text{Ct}(\text{geneB}) + \text{coef}(\text{geneC}) \times \text{Ct}(\text{geneC}) + \dots]$. In this equation, coefficients for genes that are predictors of beneficial outcome are positive numbers and coefficients for genes that are predictors of unfavorable outcome are negative numbers. The "Ct" values in the equation are ACTs, i.e. reflect the difference between the average normalized Ct value for a population and the normalized Ct measured for the patient in question. The convention used in the present analysis has been that ACTs below and above the population average have positive signs and negative signs, respectively (reflecting greater or lesser mRNA abundance). The relative risk (RR) calculated by solving this equation will indicate if the patient has an enhanced or reduced chance of long-term survival without cancer recurrence"); and

(c) using the RS to determine the likelihood of breast cancer recurrence (col 11 In 24-27).

Cobleigh also teaches the use of antibodies for screening the levels of expression of the markers (col 14, In 33-40). Although Cobleigh does not specifically disclose including the antibodies in a kit, it would have been obvious to a person skilled in the art to package the necessary detection elements disclosed by Cobleigh, such as antibodies, in a kit in order to enable simple and rapid use of the elements in diagnostic tests.

Therefore, the inventions of Groups I+ and Groups II+ lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.